

Lubricating Bacteria Model for Branching growth of Bacterial Colonies

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Abstract

Various bacterial strains (e.g. strains belonging to the genera *Bacillus*, *Paenibacillus*, *Serratia* and *Salmonella*) exhibit colonial branching patterns during growth on poor semi-solid substrates. These patterns reflect the bacterial cooperative self-organization. Central part of the cooperation is the collective formation of lubricant on top of the agar which enables the bacteria to swim. Hence it provides the colony means to advance towards the food. One method of modeling the colonial development is via coupled reaction-diffusion equations which describe the time evolution of the bacterial density and the concentrations of the relevant chemical fields. This idea has been pursued by a number of groups. Here we present an additional model which specifically includes an evolution equation for the lubricant excreted by the bacteria. We show that when the diffusion of the fluid is governed by nonlinear diffusion coefficient branching patterns evolves. We study the effect of the rates of emission and decomposition of the lubricant fluid on the observed patterns. The results are compared with experimental observations. We also include fields of chemotactic agents and food chemotaxis and conclude that these features are needed in order to explain the observations.

I. INTRODUCTION

It is now understood that the study of cooperative self-organization of bacterial colonies is an exciting new multidisciplinary field of research, necessitating the merger of biological information with the physics of non-equilibrium processes and the mathematics of non-linear dynamics. At this stage, several experimental systems have been identified, and preliminary modeling efforts are making significant progress in providing a framework for the understanding of experimental observations [1–13].

In nature bacterial colonies must often cope with hostile environmental conditions. To do so bacteria have developed sophisticated cooperative behavior and intricate communication capabilities [14–18]. These include: direct cell-cell physical interactions via extra-membrane polymers [19,20], collective production of extracellular "wetting" fluid for movement on hard surfaces [1,21], long range chemical signaling, such as quorum sensing [22–24] and chemotactic signaling¹ [25–27], collective activation and deactivation of genes [28,29,2] and even exchange of genetic material [30–32]. Utilizing these capabilities, bacterial colonies develop complex spatio-temporal patterns in response to adverse growth conditions.

For researchers in the pattern formation field, the above communication mechanisms open a new class of tantalizing complex models exhibiting a much richer spectrum of patterns than the models of non-living systems.

Fujikawa and Matsushita [4,33,34] reported for the first time² that bacterial colonies could grow elaborate branching patterns of the type known from the

¹ Chemotaxis is a bias of movement according to the gradient of a chemical agent. Chemotactic signaling is a chemotactic response to an agent emitted by the bacteria.

² We refer to the first time that branching growth was studied as such. Observations of branching colonies occurred long ago [35,36].

study of fractal formation in the process of diffusion-limited-aggregation (DLA) [37–39] . This work was done with *Bacillus subtilis*, but was subsequently extended to other bacterial species such as *Serratia marcescens* and *Salmonella anatum* [40].

Motivated by these observations, Ben-Jacob *et al.* conducted new experiments with a new species of bacteria that has been isolated from cultures of *Bacillus subtilis* [6,41,8]. The new species was designated *Paenibacillus dendritiformis* var. *dendron* [42]. This species is motile on the hard surface and its colonies exhibit branching patterns (Fig. 1). The new mode of tip-splitting growth was found to be inheritable and transferable by a single cell, hence it is referred to as a distinctive morphotype [43], and, to indicate the tip-splitting character of the growth, it was denoted \mathcal{T} morphotype. In the next section we describe in some detail the observations of Ben-Jacob *et al.* . Additional studies of branching colonial growth are reported by Matsuyama *et al.* [1,44] and Mendelson and Salhi [2].

How should one approach the modeling of the complex bacterial patterning? With present computational power it is natural to use computer models as a main tool in the study of complex systems. However, one must be careful not to be trapped in the “reminiscence syndrome”, described by J. D. Cowan [45], as the tendency to devise a set of rules which will mimic some aspect of the observed phenomena and then, to quote J. D. Cowan ”They say: ‘Look, isn’t this reminiscent of a biological or physical phenomenon!’ They jump in right away as if it’s a decent model for the phenomenon, and usually of course it’s just got some accidental features that make it look like something.” Yet the reminiscence modeling approach has some indirect value. True, doing so does not reveal (directly) the biological functions and behavior. However, it does reflect understanding of geometrical and temporal features of the patterns, which indirectly might help in revealing the underlying biological principles. Another extreme is the ”realistic modeling” approach, where one constructs an algorithm

that includes in details all the known biological facts about the system. Such an approach sets a trajectory of ever including more and more details (vs. generalized features). The model keeps evolving to include so many details that it loses any predictive power.

Here we try to promote another approach – the "generic modeling" one [46,8,47,12]. We seek to elicit, from the experimental observations and the biological knowledge, the generic features and basic principles needed to explain the biological behavior and to include these features in the model. We will demonstrate that such modeling, with close comparison to experimental observations, can be used as a research tool to reveal new understanding of the biological systems.

Generic modeling is not about using sophisticated, as it may, mathematical description to dress pre-existing understanding of complex biological behavior. Rather, it means a cooperative approach, using existing biological knowledge together with mathematical tools and synergetic point of view for complex systems to reach a new understanding (which is reflected in the constructed model) of the observed complex phenomena.

The generic models can yet be grouped into two main categories: 1. Discrete models such as the communicating walkers models of Ben-Jacob *et al.* [8,48,11] and the bions model of Kessler and Levine [46,49]. In this approach, the microorganisms (bacteria in the first model and amoebae in second) are represented by discrete, moving entities (walkers and bions, respectively) which can consume nutrients, reproduce, perform random or biased movement, and produce or respond to chemicals. The time evolution of the chemicals is described by reaction-diffusion equations. 2. Continuous or reaction-diffusion models [50,51]. In these models the microorganisms are represented via their 2D density, and a reaction-diffusion equation of this density describes their time evolution. This equation is coupled to the other reaction-diffusion equations for the chemical fields. In the context of branching growth, this idea has been pursued recently by Matsushita

et al. [52], Kawasaki *et al.* [53] and Kitsunezaki [54]. A summary and critique of this approach is provided by Rafols [55].

One of the important features of the bacterial colonies is the lubricant layer in which the bacteria swim. A model for the colony should include this feature, directly or indirectly [17,56]. Here we present a model which specifically includes the lubricant excreted by the bacteria. The model follows the second approach of generic modeling. We represent the various entities: the bacteria, the chemicals and the lubricant by continuous fields.

II. EXPERIMENTAL AND BIOLOGICAL BACKGROUND

In figure 1a we show branching patterns of bacterial colonies. Each of these colonies is made up of about 10^{10} bacteria of the type *Paenibacillus dendritiformis* var. *dendron* (see [6,41] for first reference in the literature and [42] for identification). Each colony is grown in a standard petri-dish (8cm in diameter) on a thin layer of agar (semi-solid jelly). Figure 2a shows that the branches of the colonies have well defined boundary, and the bacteria are confined by this boundary. Figures 2b and 2c highlight the constituents of the branches. Figure 2b shows that each branch is a layer of fluid on the surface of the agar. Figure 2c shows the bacteria, all of which are confined within this fluid. The bacteria cannot move on the dry surface and cooperatively they produce a layer of lubrication fluid in which they swim.

Bacterial swimming is a random-walk-like movement, in which the bacteria propel themselves in nearly straight runs separated by brief tumbling. Swimming can be done only in sufficiently inviscid fluid. To produce such fluid the bacteria secrete lubricant (wetting agents). Other bacterial species produce known extracellular lubricants (also known as surfactants, see [1,57,58] and references therein). These are various materials (various cyclic lipopeptides were identified) which draw water from the agar. The composition and properties of the lubricant

of *P. dendritiformis* var. *dendron* is not known, but we will assume that higher concentration of lubricant is needed to extract water from a dryer agar, and that the lubricant is slowly absorbed into the agar (or decomposes).

In order to move, reproduce and perform other metabolic activities, the bacteria consume nutrients from the media, nutrients which are given in limited supply. The growth of a colony is limited by the diffusion of nutrients towards the colony – the bacterial reproduction rate that determines the growth rate of the colony is limited by the level of nutrients available for the cells. If nutrient is deficient for a long enough period of time, the bacteria may enter a pre-spore state, i.e. begin the process of sporulation. They stop normal activity – like movement – and use all their internal reserves to metamorphose from an active volatile cell to a spore – sedentary durable “seed”. The sporulating bacteria emit a wide range of materials, some of which unique to the sporulating bacteria. These emitted chemicals might be used by other bacteria as a signal carrying information about the conditions at the location of the pre-spores.

The patterns in figure 1a are arranged in a diagram according to two control parameters: the initial concentration of nutrients (horizontal axis, increasing from left to right), and the concentration of the agar, or the dryness of the media (vertical axis, increasing from bottom to top). The chirality of the colonies at the top row is due to interaction between repulsive chemotaxis (see Sec. V) and the process of tumbling (see Refs. [48,16] for details). It will be ignored in this paper. For high level of initial nutrients concentration (right column), the patterns are compact, with wide branches. For intermediate levels of nutrients, the lower the initial concentration is, the more ramified and less ordered the patterns are. The patterns become fractal-like, with fractal dimension decreasing for lower levels of nutrients. For the same nutrient level, higher agar concentration makes the pattern less dense, with larger gaps between the branches. All of the above phenomena could be expected from our knowledge on patterning in non-living systems [59–62,16]. Unlike what could have been expected, at the lowest

concentration of nutrient (leftmost column), the pattern are more ordered with a well defined circular envelope. This phenomenon demonstrates the complexity of the biological system, and its explanation needs an additional biological feature – chemotaxis signaling (see section V). Figure 3 demonstrates that in spite of this complexity and the inherit noise in the system, the experiments are controlled enough for the patterns to be reproducible.

III. CONSTRUCTION OF THE MODEL

The model includes four coupled fields projected on 2D. One field describes the density of motile bacteria $b(\vec{x}, t)$, the second describes the height of the lubricant layer in which the bacteria swim $l(\vec{x}, t)$, third field describes the concentration of nutrients $n(\vec{x}, t)$ and the fourth field is the density of stationary bacteria $s(\vec{x}, t)$, bacteria that enter the pre-spore state.

We first describe the dynamics of the bacteria and of the nutrient. The two reaction-diffusion equations governing those fields have the following form:

$$\begin{aligned} \frac{\partial b}{\partial t} &= \textit{movement} + \textit{reproduction} - \textit{sporulation} \\ \frac{\partial n}{\partial t} &= \textit{diffusion} - \textit{consumption} \end{aligned} \tag{1}$$

The sporulation term refers to the transition of motile bacteria into the stationary state, i.e. the pre-spore state. The nutrient diffusion is a simple diffusion process with a constant diffusion coefficient. The rate of nutrient consumption is proportional to the rate of bacterial reproduction. For the reproduction term in (1) we take:

$$\textit{reproduction} = knb \tag{2}$$

where k is a constant rate. This is the usual form included in reaction-diffusion models, but see section VI for more detailed discussion. The exact form of the sporulation term is not known. For simplicity we take the form:

$$sporulation = \mu b \quad (3)$$

where the rate μ is constant.

We now turn to the bacterial movement. In a uniform layer of liquid, bacterial swimming is a random walk with variable step length and can be approximated by diffusion. The layer of lubricant is not uniform, and its height affects the bacterial movement. An increase in the amount of lubricant decreases the friction between the bacteria and the agar surface. The term 'friction' is used here in a very loose manner to represent the total effect of any force or process that slows down the bacteria. It might include, for example, the drag which acts on a body moving in shallow layer of viscous fluid. It might include the probability that a flagellum will adhere or get tangled with the polymers of the agar. We suggest that the bacterial movement depends on the local lubricant height through a power law with the exponent $\gamma > 0$:

$$movement = \nabla \cdot (D_b(l/l_M)^\gamma \nabla b) \quad (4)$$

where D_b is a constant with dimensions of a diffusion coefficient and l_M is the height preferred by the bacteria (see below). D_b is related to the fluid's viscosity and the dryness of the agar might affect this viscosity. But as we argue below, this is probably not the main influence of the agar dryness.

Gathering the various terms gives the partial model:

$$\begin{aligned} \frac{\partial b}{\partial t} &= \nabla \cdot (D_b(l/l_M)^\gamma \nabla b) + kbn - \mu b \\ \frac{\partial n}{\partial t} &= D_n \nabla^2 n - \alpha kbn \\ \frac{\partial s}{\partial t} &= \mu b \end{aligned} \quad (5)$$

where α is a conversion factor, being the amount of nutrient consumed for reproduction of a new bacterium. The third equation in (5) describes the stationary bacteria. Since they are immotile their dynamics include only a source term: their conversion from the motile state.

The dynamics of the lubricating fluid are also governed by a reaction diffusion equation. There are two reaction terms: production by the bacteria and absorption into the agar. The dynamics of the field are given by:

$$\frac{\partial l}{\partial t} = -\nabla \cdot \vec{J}_l + f_l(b, n, l) - \lambda l \quad (6)$$

where \vec{J}_l is the fluid flux (to be discussed), $f_l(b, n, l)$ is the fluid production term and λ is the absorption rate of the fluid into the agar.

We assume that the fluid production depends on the bacterial density. As the production of lubricant probably demands substantial metabolic efforts, it should also depend on the nutrient's level. We take the simplest case where the production depends linearly on the concentrations of both the bacteria and the nutrients. We presume that the bacteria produce lubricant only when it is needed, i.e. when its height is below a threshold height, denoted as l_M . We therefore take the production term to be:

$$f_l(b, n, l) = \Gamma b n (l_M - l) \quad (7)$$

where Γ is the production rate.

We turn to the flux of the lubricating fluid. The lubricating fluid flows by diffusion and by convection caused by bacterial motion. A simple description of the convection is that as each bacterium moves, it drags along with it the fluid surrounding it.

$$\vec{J}_l = -D_l (l/l_M)^\nu \nabla l + j \vec{J}_b \quad (8)$$

where D_l is a constant with dimensions of a diffusion coefficient, \vec{J}_b is the bacterial flux and j is the amount of fluid dragged by each bacterium. The diffusion term of the fluid depends on the height of the fluid to the power $\nu > 0$ (the nonlinearity in the diffusion of the lubricant, a very complex fluid, is motivated by hydrodynamics of simple fluids). The nonlinearity causes the fluid to have a sharp boundary at the front of the colony, as is observed in the bacterial colonies. The equation for the lubricant field is:

$$\frac{\partial l}{\partial t} = \nabla \cdot (D_l(l/l_M)^\nu \nabla l + j D_b(l/l_M)^\gamma \nabla b) + \Gamma b n(l_M - l) - \lambda l \quad (9)$$

The functional form of the terms that we purposed are simple and plausible, but they were not derived from basic physical principles. Therefore we cannot derive exact relations between the parameters of those terms and the physical properties of the agar substrate. However we can propose some relations between the parameters and the agar. In the experiments, the agar concentration is controlled. Higher agar concentration gives a drier and more solid substrate. The lubricating fluid is composed of lubricant and water. Increasing the agar concentration has several effects on the fluid and its dynamics. Since the agar will be drier the absorption rate λ will increase. It will also make it more difficult for the lubricant to extract water from the agar substrate. Therefore the production rate Γ should decrease. More lubricant will be required to extract water so the composition of the fluid will change. The lubricant concentration will increase, making the fluid more viscous, thus slowing its flow. Therefore the diffusion coefficient D_l should decrease. A viscous fluid will also slow the bacterial movement, so the bacterial diffusion coefficient D_b should also decrease.

Equation (9) together with equations (5) form our model. Before further studies of the model we reduce the number of parameters by using dimensionless units. We define the new variables:

$$\begin{aligned} t' &= t\mu, & \vec{x}' &= \vec{x}\sqrt{\mu/D_n}, \\ b' &= bk\alpha/\mu, & n' &= nk/\mu, & l' &= l/l_M \end{aligned} \quad (10)$$

With the same units we define:

$$\begin{aligned} D'_b &= D_b/D_n, & D'_l &= D_l/D_n, \\ \Gamma' &= \Gamma\mu/k^2\alpha, & \lambda' &= \lambda/\mu, & j' &= j\mu/k\alpha l_M \end{aligned} \quad (11)$$

Using these variables in (5) and (9) and omitting the primes we get:

$$\frac{\partial b}{\partial t} = \nabla \cdot (D_b l^\gamma \nabla b) + b n - b$$

$$\begin{aligned}
\frac{\partial n}{\partial t} &= \nabla^2 n - bn \\
\frac{\partial l}{\partial t} &= \nabla \cdot (D_l l^\nu \nabla l + j D_b l^\gamma \nabla b) + \Gamma b n (1 - l) - \lambda l \\
\frac{\partial s}{\partial t} &= b
\end{aligned} \tag{12}$$

IV. RESULTS OF NUMERIC SIMULATION

Figures 4-10 show results of numerical simulations of the model. The figures display the sum of the active and stationary bacterial densities, $b + s$. The simulation were done with an explicit method. To reduce the implicit lattice anisotropy, a quenched noise was introduced into the diffusion operators. For the initial conditions, we set n to have uniform distribution of level n_0 , b to be zero everywhere but in the center, and the other fields to be zero everywhere.

In figure 4 we show the fields s , n , b and l . The nutrient field n was consumed by bacteria down to a level of $n \sim 1$ (μ/k in the original units) in the area covered by the colony (the nutrient was not completely depleted due to the functional form of the bacterial sporulation term, the same as in [54] and unlike [53,52]). As the nutrient diffuses faster than the other fields it also decreased in the area between the colony branches. The field of the motile bacteria b and the lubricant field l are overlapping. The motile bacteria are confined to the area covered by lubricant. The fronts of both fields have compact support as figure 5 shows.

In experiments of the bacterial colonies there are two control parameters: the agar concentration and the initial nutrient concentration. First we examined the effect of changing the latter. As figure 6 shows, we obtained a dense circular colony when n_0 was large, a branched pattern when we decreased n_0 and a DLA-like pattern when n_0 was close to 1. Similar effects of decreasing the initial nutrient level appear in other reaction-diffusion models [52–54,56].

Changing the agar concentration affects the dynamics of the lubricant fluid. We suggested in the previous section that a higher agar concentration relates to a

larger absorption rate λ and to lower production rate Γ and diffusion coefficients D_l and D_b . It is not a-priori clear what is the exact dependence of each parameter on the agar concentration. We use the model to investigate this question. In figure 7 we show patterns obtained with different values of the parameters Γ and λ . As we expected, increasing λ or decreasing Γ produced a more ramified pattern, similar to the effect of higher agar concentrations on the patterns of bacterial colonies (figure 1a). The value of the diffusion coefficient of the lubricating fluid D_l has less influence on the colony pattern, as can be seen in figure 8. In contrast, decreasing the bacterial diffusion coefficient D_b produces a ramified pattern, as figure 9 shows.

In most of the figures we took $\gamma = 1$ and $\nu = 1$. There is no a-priori reason to take these values, and in figure 10 we show the effect of other values on the growth. While the patterns are different, we found that these changes can be compensated by other parameters and varying γ and ν has no qualitative effect on the conclusions.

V. CHEMOTAXIS

The model so far reproduced most of the features of the experimental results displayed in figure 1a, but does not reproduce the transition to ordered patterns at the lowest nutrient concentration. We will now extend the lubricant model to test for its success in describing this phenomenon. Ben-Jacob *et al.* suggested that this transition is due to chemotaxis and chemotactic signaling [63–65,16]. Chemotaxis means changes in the movement of the cell in response to a gradient of certain chemical field [66–69]. The movement is biased along the gradient either in the gradient direction or in the opposite direction. Usually chemotactic response means a response to an externally produced field, like in the case of chemotaxis towards food. However, the chemotactic response can be also to a field produced directly or indirectly by the bacterial cells. We will refer to this

case as chemotactic signaling.

We incorporate the effect of chemotaxis by introducing a bacterial flux \vec{J}_{chem} due to chemotaxis. The general form is:

$$\vec{J}_{chem} \equiv \zeta(l)b\chi(r)\nabla r \quad (13)$$

where r is the chemotactic chemical. $\chi(r)\nabla r$ is the gradient sensed by the bacterium (with $\chi(r)$ having the units of 1 over chemical's concentration). $\chi(r)$ is taken to be the “receptor law”, i.e. $\chi(r) = K_r/(K_r + r)^2$ [70]. K_r is a constant that determines the concentration range of the chemical for which the chemotaxis is effective. $\zeta(l)$ (having the same units as a diffusion coefficient) is the bacterial response to the sensed gradient (i.e. the effect on the bacterial movement). In our model the bacterial diffusion coefficient is $D_b l^\nu$, and the bacterial response to chemotaxis is $\zeta(l) = \zeta_0 (D_b l^\nu)$. ζ_0 is a constant, positive for attractive chemotaxis and negative for repulsive chemotaxis.

Amplification of diffusive Instability Due to Nutrients Chemotaxis: In non-living systems, more ramified patterns (lower fractal dimension) are observed for lower growth velocity. Based on growth velocity as function of nutrient level and based on growth dynamics, Ben-Jacob *et al.* [8] concluded that in the case of bacterial colonies there is a need for mechanism that can both increase the growth velocity and maintain, or even decrease, the fractal dimension. They suggested food chemotaxis to be the required mechanism. It provides an outward drift to the cellular movements; thus, it should increase the rate of envelope propagation. At the same time, being a response to an external field it should also amplify the basic diffusion instability of the nutrient field. Hence, it can support faster growth velocity together with a ramified pattern of low fractal dimension. The bacterial flux due to nutrient chemotaxis is:

$$\vec{J}_{nutrient} \equiv \zeta_n D_b l^\nu \chi_n b \frac{K_n}{(K_n + n)^2} \nabla n \quad (14)$$

In figure 11 it is shown that as expected, the inclusion of food chemotaxis led

to a considerable increase of the growth velocity without significant change in the fractal dimension of the pattern.

Repulsive chemotactic signaling: We focus now on the formation of the fine radial branching patterns at low nutrient levels. From the study of non-living systems, it is known that in the same manner that an external diffusion field leads to the diffusion instability, an internal diffusion field will stabilize the growth. It is natural to assume that some sort of chemotactic agent produces such a field. To regulate the organization of the branches, it must be a long-range signal. To result in radial branches it must be a repulsive chemical produced by bacteria at the inner parts of the colony. The most probable candidates are the bacteria entering a pre-spore state, which were referred to as the stationary bacteria s .

As stated above, bacteria may enter a pre-spore state upon starvation. In this process they emit a wide range of waste materials, some of which unique to the sporulating bacteria. These emitted chemicals might be used by other bacteria as a signal carrying information about the conditions at the location of the pre-spores. Ben-Jacob *et al.* [8,71,64] suggested that such materials are repelling the bacteria ('repulsive chemotactic signaling') as if they escape a dangerous location.

The equation describing the dynamics of the chemorepellent contains terms for diffusion, production by pre-spores, decomposition by active bacteria and spontaneous decomposition:

$$\frac{\partial r}{\partial t} = D_r \nabla^2 r + \Gamma_r s - \Omega_r b r - \lambda_r r \quad (15)$$

where D_r is the diffusion coefficient of the chemorepellent, Γ_r is the emission rate of repellent by pre-spores, Ω_r is the decomposition rate of the repellent by active bacteria, and λ_r is the rate of self decomposition of the repellent. The bacterial flux due to repulsive chemotaxis is:

$$\vec{J}_{repulsive} \equiv \zeta_r D_b l^\nu \chi_r b \frac{K_r}{(K_r + r)^2} \nabla r \quad (16)$$

where $\zeta_r < 0$ (repulsive chemotaxis).

In figure 12 the effect of repulsive chemotactic signaling is shown. In the presence of repulsive chemotaxis the patterns have a smooth circular envelope, while the branches are thinner and radially oriented.

VI. ANOTHER LOOK ON MODELING BACTERIAL GROWTH

The model presented in section (III) goes along the lines of existing models [54,52] in interpreting the bacterial growth terms (reproduction and sporulation). There are, however, some biological consideration that may have been overlooked. In section III we termed knb as 'reproduction' and μb as 'sporulation'. But looking at them term by term unravel discrepancies. The so called "sporulation" term in the equations represents, according to the usual interpretation, a constant probability per time unit for a bacterium to enter the pre-spore state. This is incompatible with our biological knowledge. A nutritional stress is one of the pre-requirements for starting the process of sporulation. As long as there is enough food for the bacteria to reproduce, there is no nutritional stress. In the model it means that as long as the (local) bacterial density increases (i.e. as long as the sum of all the reaction terms is positive) there can be no sporulation.

This leads to a new interpretation of the reaction terms; the resources that the bacteria consume from the nutrient (energy and materials) are utilized for two main processes: to sustain life and to reproduce. Therefore the reproduction rate should be proportional to the nutrient consumed less the amount required for life sustaining activities. We assume that the latter is required by each bacterium at a constant rate, independent of the nutrient level or the bacterial density. We denote that rate by μ , which previously denoted the sporulation rate. Then we have instead of (2), with $g(b, n)$ denoting the nutrient consumption rate:

$$reproduction = g(n, b) - \mu b \tag{17}$$

$$sporulation = \begin{cases} 0 & \text{if } reproduction > 0 \\ -reproduction & \text{if } reproduction < 0 \end{cases} = \max(\mu b - g(n, b), 0) \tag{18}$$

So far we have not changed much the model. All the terms involved in the dynamics remain the same, but we give them new interpretations. Only the dynamics of the field s changed. Since the dynamics of the other fields are decoupled from the field s , the modified model is effectively identical to the previous model.

As we changed the meaning of the bacterial growth terms, we should reconsider their functional form, focusing our attention on the nutrient consumption term, $g(n, b)$. The term $g(n, b) = knb$ was taken to be the limit of growth at low nutrient concentration, but it is not a-priori evident. We suggest that g should have three regimes corresponding to different limits of the nutrient concentration and bacterial density.

1. There is a maximal growth rate of the bacteria, along with a maximal rate of nutrient consumption, even for optimal conditions. We denote the maximal consumption rate per bacterium by Ω_n .
2. When conditions are not optimal and the nutrient concentration is low, it might be the limiting factor. Nutrient consumption is then diffusion-limited and its rate is proportional to $D_n n$ per bacterium, as D_n defines the effective area from which a bacterium consumes food (assuming no other bacteria interfere with this process).
3. When the nutrient concentration is low and the bacterial density is high, there is competition between the bacteria, and the amount of nutrient available for each bacterium is proportional to n/b .

As can be seen in figure 2c, the density of bacteria can be quite high and so the third limiting behavior cannot be ignored. The ratio between the nutrient diffusion length and the distance between bacteria $b^{-1/2}$ determines which is the appropriate regime. We take the nutrient consumption rate to be the minimum of the three expressions:

$$g(b, n) = b \min(\Omega_n, kn, en/b) \quad (19)$$

where $k = D_n/\alpha$ and e is a constant rate (the min function could have been replaced by a smooth function with the same limits, but we will put it in this way for clarity). We note that the Michaelis-Menten law [70] of $\frac{k}{1+gn}nb$ as the first two expressions as limits, but does not incorporate the third one. The new model in dimensionless form is:

$$\begin{aligned} \frac{\partial b}{\partial t} &= \nabla \cdot (D_b l^\gamma \nabla b) + g(n, b) - b \\ \frac{\partial n}{\partial t} &= \nabla^2 n - g(n, b) \\ \frac{\partial l}{\partial t} &= \nabla \cdot (D_l l^\nu \nabla l + j D_b l^\gamma \nabla b) + \Gamma b n (1 - l) - \lambda l \\ \frac{\partial s}{\partial t} &= \max(b - g(n, b), 0) \end{aligned} \quad (20)$$

where $g(n, b) = b \min(\frac{\Omega_n}{\mu}, n, \frac{e\alpha}{\mu} \frac{n}{b})$. The same transformation of variables (10) and (11) were employed to obtain the dimensionless model.

Numerical simulations of the above model show (figure 13) no qualitative differences from the model presented in section IV. It is not the mathematical model which is important in this respect, but our understanding of the biological system. This emphasizes the differences between the 'generic modeling approach' and the approach driving the "reminiscence syndrome"

VII. CONCLUSION

We first briefly reviewed experimental observations of branching patterns in bacteria of the species *Paenibacillus*. Both colonial patterns and optical microscope observations of the bacteria dynamics were presented.

Our goal in this manuscript was to test a new reaction-diffusion model which includes time evolution equation of a lubricant. From a comparison of the model simulation and experimental observations we conclude that when a specific bacterial strain is considered, such comparison is not sufficient to tell us if indeed the right biological features are included in the model.

For more critique test of the models, additional aspects of the growth (such as functional dependence of the colonial growth velocity on growth conditions, branches size and width distributions etc.) have to be compared with the model predictions. One should also compare the theory with more involved experimental tests, such as the effect of imposed anisotropy, competition between neighboring colonies, and expression of mutants (emergence of sectors) in expanding colonies.

Our conclusion from the study of bacterial branching growth is that the minimal features of diffusion, food consumption, reproduction and inactivation are not sufficient to explain the complete picture of the observed phenomena. We believe that additional mechanisms must be introduced, and propose chemotactic signaling as plausible one.

This work has dealt with a continuous model. Such models are not preferable to discrete ones. Each has its advantages and disadvantages. The discrete walkers model, for example, enables us to include the valuable feature of internal degrees of freedom, but is computationally limited in the number of walkers that can be simulated, and thus its scaling to the real problem is somewhat problematic. The best strategy is to employ in parallel both the reaction-diffusion and the walkers approaches.

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FIGURES

FIG. 1. a) A diagram of colonial branching patterns of the bacteria *Paenibacillus dendritiformis* var. *dendron*. The dot at the center of each colony is the initial inoculum. The horizontal axis of the diagram is initial nutrient concentration. From left to right (in units of *gram/liter*) it is 0.1, 0.5, 1.0, 2.0 and 3.0 . The vertical axis is agar concentration. From bottom to top (in units of *10gram/liter*) it is 1.5, 2 and 2.5 . As the level of initial nutrient concentration is decreased the patterns become less organized with fewer branches. However, at the lowest concentration, of 0.1*g/l*, the pattern is ordered with circular envelope. b) An isolated example of branching pattern at 0.5*g/l* pepton and 1.75% agar concentration.

FIG. 2. Closer look on branches of a colony. a) $\times 20$ magnification shows the sharp boundaries of the branches. The width of the boundary is in the order of micron. b) Numarsky (polarized light) microscopy shows the hight of the branches and their envelope. What is actually seen is the layer of lubrication fluid, not the bacteria. c) $\times 50$ magnification shows the bacteria inside a branch. Each bar is a single bacterium. There are no bacteria outside the branch.

FIG. 3. Demonstration of the reproducibility of the colonial patterns. Four colonies in the same plate (from four inocula).

FIG. 4. A representative branching pattern produced by the lubricating bacteria model. The image shows the four fields of the model.

FIG. 5. Profile of the fronts of the bacterial field (solid line) and of the lubricating field (dashed line) from the 2D model. The fields propagate to the right. Both fronts have compact support.

FIG. 6. Effect of varying the initial nutrient concentration n_0 on colony pattern. The minimal value of n_0 to support growth is 1. We include the time t it took for the colony to grow.

a. $n_0 = 1.1, t = 38089$, DLA-like pattern.

b. $n_0 = 1.3, t = 11410$.

c. $n_0 = 1.7, t = 3671$, branched pattern.

d. $n_0 = 3, t = 721$, dense branches.

e. $n_0 = 5, t = 216$, a disk.

The other parameters are: $D_b = D_l = 0.5, \Gamma = 0.6, \lambda = 0.1, j = 0.01, \gamma = 1, \nu = 2$.

FIG. 7. Effect of varying λ , the fluid absorption rate, on colony pattern. The fluid production rate Γ is 1 in the upper row, and 0.3 in the lower row. In both rows λ increases from left to right: $\lambda = 0.03$ (left), $\lambda = 0.1$ (center), $\lambda = 1$ (right). The patterns become more ramified as λ increases. Here and in figures 8, 9 decreasing Γ also produces a more ramified pattern. The other parameters are: $D_b = D_l = 1, \gamma = \nu = 1, n_0 = 1.5, j = 0.01$

FIG. 8. Effect of varying D_l , the fluid diffusion coefficient, on colony pattern. Fig. (a) and (d) are the same as Fig. 7 (a) and (d). In both rows D_l decreases from left to right: $D_l = 1$ (left), $D_l = 0.1$ (center), $D_l = 0.01$ (right). The patterns do not change significantly.

FIG. 9. Effect of varying D_b , the bacterial diffusion coefficient, on colony pattern. Fig. (a) and (d) are the same as Fig. 7 (a) and (d). In both rows D_b decreases from left to right: $D_l = 1$ (left), $D_l = 0.1$ (center), $D_l = 0.05$ (right). The patterns become more ramified as D_b decreases.

FIG. 10. Effect of changing the exponents γ and ν . We also print the growth time of the colony t . The parameters of the figures are:

- a. $\gamma = 1, \nu = 1, t = 1249$.
- b. $\gamma = 1, \nu = 2, t = 2069$.
- c. $\gamma = 2, \nu = 1, t = 4451$.
- d. $\gamma = 2, \nu = 2, t = 10066$.

The other parameters are: $D_b = D_l = 1, n_0 = 1.5, \Gamma = 1, \lambda = 0.03, j = 0.01$.

FIG. 11. The effect of food chemotaxis on growth. The four patterns differ in the values of ζ_n , the response to the sensed gradient of the nutrient.

We also print the growth time of the colony t .

- a. $\zeta_n = 0, t = 5101$ (no chemotaxis).
- b. $\zeta_n = 10, t = 4957$.
- c. $\zeta_n = 30, t = 3843$.
- d. $\zeta_n = 100, t = 2053$.

The other parameters are: $D_b = D_l = 1, \gamma = \nu = 1, n_0 = 1.5, \Gamma = 0.3, \lambda = 0.1, j = 0$.

FIG. 12. The effect of repulsive chemotaxis on growth. The four patterns differ in the values of ζ_r , the response to the sensed gradient of the chemorepellent.

- a. $\zeta_r = 0$ (no chemotaxis).
- b. $\zeta_r = -20$.
- c. $\zeta_r = -50$.
- d. $\zeta_r = -100$.

The other parameters are: $D_b = D_l = 1, \gamma = \nu = 1, n_0 = 1.3, \Gamma = 0.3, \lambda = 0.1, j = 0$.

FIG. 13. Pattern produced by the modified model of section VI

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